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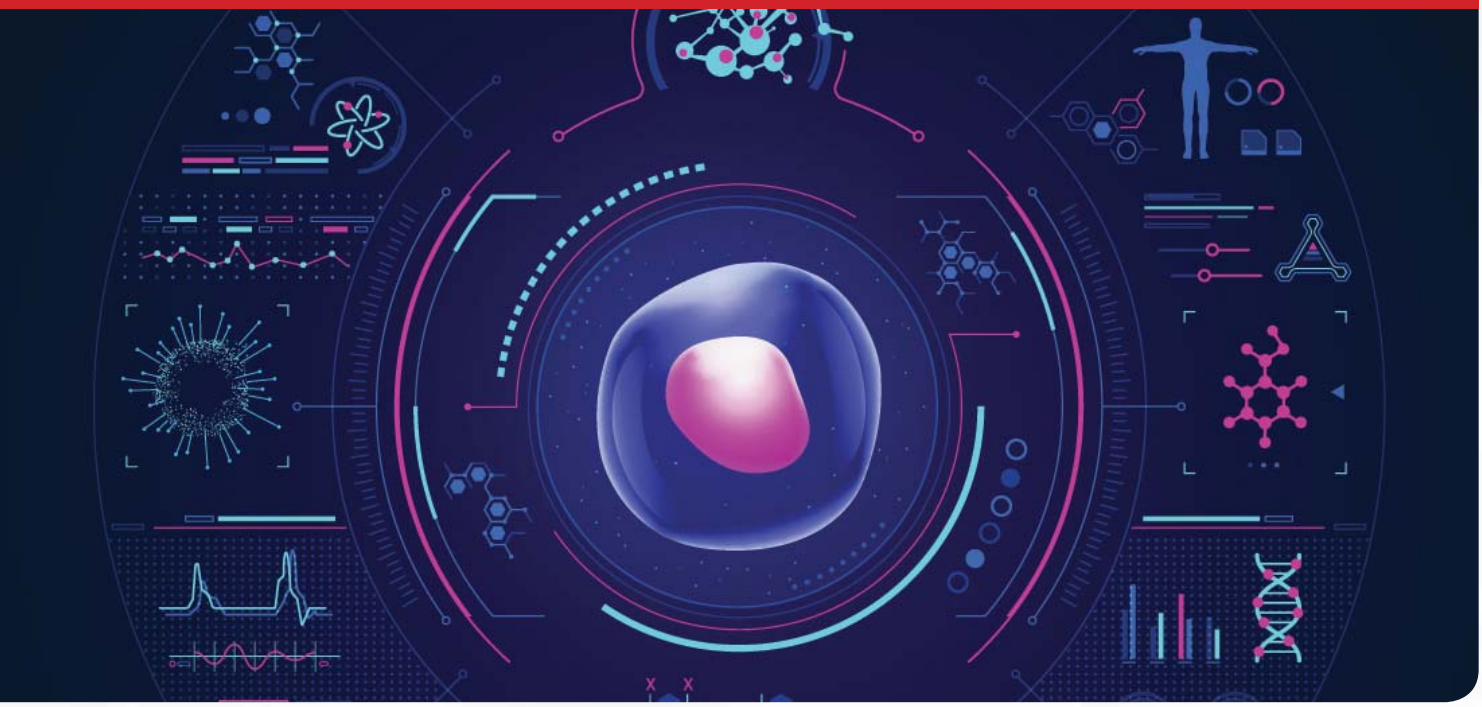
GENE AND CELL THERAPIES FOR CANCER



INTRODUCTION

The basic definition of gene therapy is the correction of a genetic defect by editing or replacing the mutated genetic material. Most of the initial gene therapy development focus was on rare monogenic diseases where a known defect is targeted and the biological and clinical endpoints are well defined. In contrast, cancer is a genetic disease that has a multi-stage development involving multiple somatic and inherited mutations. Additionally, there is known heterogeneity within tumors that can impact the efficacy of gene therapies. However, there have been significant scientific and technological developments that have resulted in the approval of oncolytic virus and cell-based therapies for specific cancer types and there is a strong preclinical and clinical pipeline for gene and cell therapies targeting various tumor types.

ONCOLYTIC VIRUSES

Gene therapy modalities fall into two main categories – viral vectors and non-viral modalities and both approaches have advantages and challenges. Viral vectors also called oncolytic viruses that efficiently infect various types of tumor cells and express high levels of payload proteins. Oncolytic viruses range from large DNA viruses such as vaccinia to small RNA viruses such as poliovirus and have several characteristics that are well suited to trigger direct tumor killing (oncolysis) or immune-mediated tumor killing[1]. Oncolytic viruses have a range of capacity to accommodate transgenes in their genomes.

For example, adeno-associated viruses can package 4 kb of foreign genetic material while herpes simplex virus type 1 (HSV1) can package about 30 kb of foreign material. Oncolytic viruses can be engineered to selectively infect tumor cells and reduce neutralizing immune responses. The viruses typically bind cell-surface receptors and are then internalized where they replicate and release particles to infect other tumor cells. Tumor cell killing takes place in 2 ways – direct oncolysis where the release of viral particles lyses the cell and immune-mediated cell killing. After infection, oncolysis occurs first and triggers the activation of an antitumor response. The recognition and killing of tumor cells are primarily mediated by natural killer or NK cells and tumor antigen-specific cytotoxic T lymphocytes. Essentially, oncolytic viruses can directly kill infected tumor cells and can also trigger an inflammatory response that results in more widespread tumor cell killing. Oncolytic viruses can also be combined with checkpoint inhibitors and other immunomodulatory therapies. Currently, immunomodulatory therapies have limited benefits in patients with immunologically "cold" tumors, so the combination of oncolytic viruses with checkpoint inhibitors is being investigated in clinical trials. The rationale behind this approach is to first stimulate an inflammatory response and turn a cold tumor "hot" with the oncolytic virus, so that the checkpoint inhibitor can induce an antitumor immune response. There are over 100 clinical trials ongoing using oncolytic viruses as monotherapies or as a part of a rational combination. To date, one therapy has been approved[2] – Imlygic® (talimogene laherparepvec), an engineered HSV1 that expresses GM-CSF, that is approved for the treatment of metastatic melanoma. Oncolytic viruses are administered using various routes: direct injection into the tumor (intratumoral) is widely used but increasingly there is interest in more systemic administration[1] such as intravenous or localized administration such as injection into the tumor bed after surgical resection. Another area of interest is the induction of a bystander effect where tumor cells adjacent to the infection site are killed and abscopal effect where tumors at distant sites are infected by the oncolytic virus. The induction of bystander and abscopal effects hold promise that oncolytic viruses can be used in late-stage metastatic cancers.

NON-VIRAL MODALITIES

Non-viral modalities are another approach to delivering genes to a tumor and are considered to be safer than viral vectors, but not as efficient[3]. Several non-viral based methods to deliver genes are being studied, but due to efficiency concerns, this area is not as advanced as viral vectors. Some of the methods to deliver include liposomes, nanoparticles and dendrimers are being studied but at this time, there is limited interest in using non-viral vectors for gene therapy in cancer. Preclinical studies in animal models using non-viral vectors have highlighted the need for multiple doses directly to the tumor to improve efficiency and increase payload expression[3]. Given the limitation of non-viral vector spread through a tumor, it is essential to dose frequently and use payloads that change the tumor microenvironment.

STRATEGIES IN CANCER GENE THERAPIES:

The rational design of a strategy to deliver the appropriate transgene or inhibitor requires an understanding of the specific tumor biology and identify the disease driver mutations. There are four broad categories of therapeutic interventions[4]:

- Deliver a gene to induce regulated cell death in tumors or increase sensitivity to chemotherapy/ radiation therapy or induce cytotoxicity.
- Deliver a normal copy of a tumor suppressor gene to replace a mutated or silenced gene
- Deliver an antisense RNA or DNA to inhibit oncogene expression
- Enhance immune cell recognition of a tumor by changing the tumor microenvironment

Regulated cell death has expanded beyond programmed cell death or apoptosis and now includes necroptosis (regulated necrosis), ferroptosis (iron-dependent regulated necrosis), autophagy-dependent cell death and alkaliptosis (pH dependent regulated necrosis)[5]. All these forms of cell death have been investigated as methods to kill tumors. The most well studied though is apoptosis induced by genes associated with the TNF (tumor necrosis factor) signaling pathway. The *MDA-7* or *IL-24* gene has been shown to selectively induce apoptosis in cancer cells but not normal cells and is under investigation as a broad-spectrum antitumor gene therapy[6]. Another strategy is to convert prodrugs into active agents by introducing the required enzyme using a viral vector. The most well-known example is using HSV1 expressing thymidine kinase followed by treatment with ganciclovir.

Families of tumor suppressors have been intensively investigated as therapeutic agents and p53 is likely one of the best studied tumor suppressor proteins. In 2003, Gendicine, an adenovirus expressing p53, became the first approved gene therapy for cancer, when it was approved in China for the treatment of head and neck squamous cell cancer[7]. Unfortunately, the FDA did not approve Advexin which was also an adenovirus expressing p53 due to safety concerns[8].

Antisense oligonucleotides are being widely used in various disease areas including cancer[9]. Custirsen is an antisense oligonucleotide that inhibits TRPM-2 (testosterone-repressed prostate message-2), an anti-apoptotic protein that is overexpressed in prostate cancer cells and is associated with resistance

to chemotherapies. Unfortunately, custirsen failed to prolong overall survival in a phase III prostate cancer trial when used in combination with docetaxel and prednisone[10]. AZD9150 is another antisense oligonucleotide that inhibits STAT3 protein expression leading to apoptosis in tumor cells. Currently, AZD9150 is in clinical trials in combination with durvalumab targeting PD-L1 and chemotherapies[11]. Using microRNAs (miRNA) as diagnostic tools is an area of active research and a microRNA panel is currently being used to differentiate benign and malignant thyroid nodules[12].

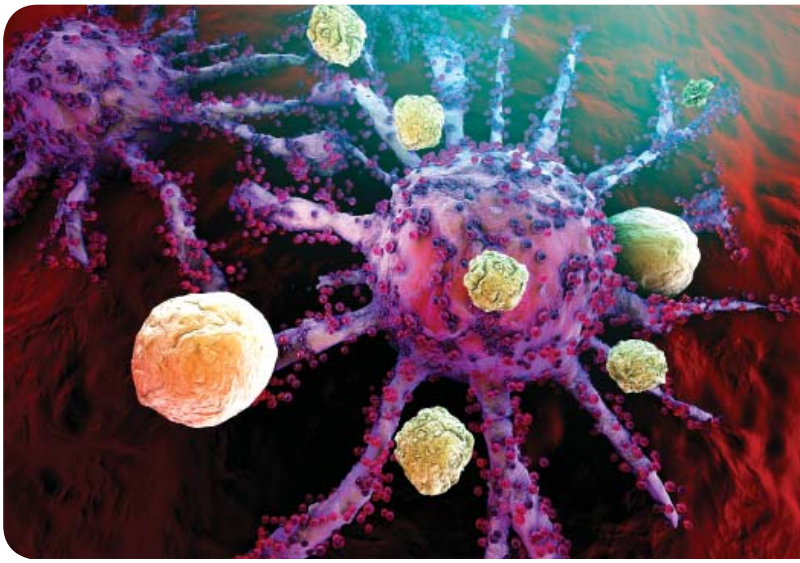
Reprogramming the tumor microenvironment (TME) to lift immunosuppression is one of the most active areas in oncology drug development. The TME includes extracellular matrix, stromal cells including fibroblasts and vascular cells and immune cells (including T and B cells, NK cells and tumor-associated macrophages). As tumors grow, the TME is remodeled and becomes dense and stiff, resulting in desmoplasia. Additionally, the TME is highly inflamed and has both innate and adaptive immune cells in the environment. Depending on the tumor, immune cells may be at the periphery of the tumor or infiltrated within the tumor. Several strategies are being investigated to harness the immune system to tackle tumor growth including targeting the chronic inflammation state and activating NK and CD8+ T cells or overexpressing GM-CSF to increase antibody dependent cytotoxicity[13]. GM-CSF is the payload for the approved oncolytic virus Imlygic® and is also being used in other gene therapies that are in clinical trials.

The most well-known therapies are immune checkpoint inhibitors which are monoclonal antibodies that block the immunoregulatory signals delivered by tumor cells to cytotoxic T-cells. Ipilimumab(anti-CTLA4) and Nivolumab and Pembrolizumab (anti-PD-1) are examples of successful immune checkpoint inhibitors. Viral vectors expressing checkpoint inhibitors or immunostimulatory proteins are being developed to target the TME remodeling along with other payloads including enzymes like hyaluronidase that degrade specific ECM components to improve delivery to desmoplastic tumors.

CELLULAR IMMUNOTHERAPIES IN CANCER

Adoptive cell therapy is an approach to harness a patient's immune cells to induce an antitumor response. There are four categories of adoptive cell therapeutic strategies:

- Tumor-infiltrating lymphocytes
- Engineered T Cell Receptors (TCR)
- Chimeric Antigen Receptor (CAR)- T cells
- Natural Killer (NK) cells

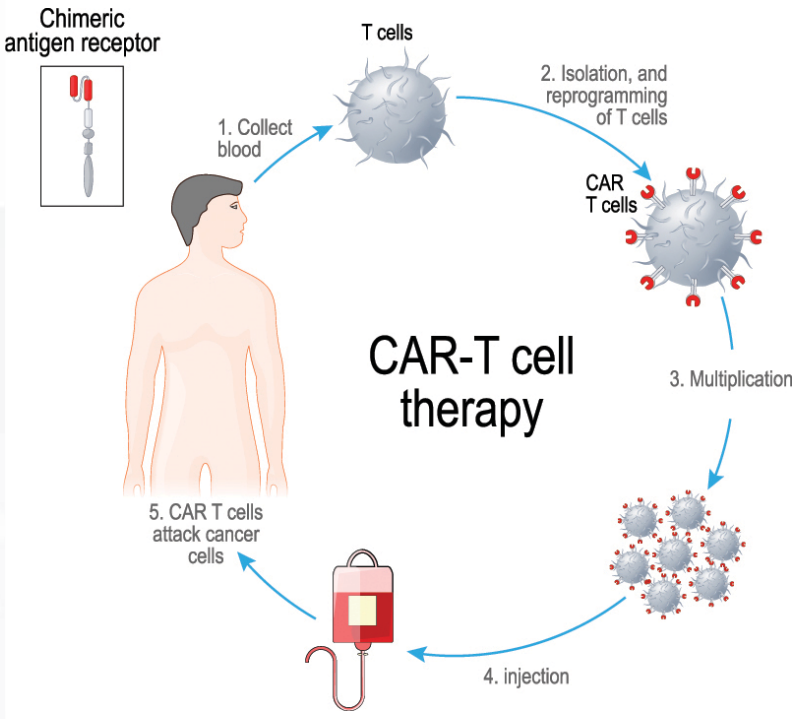


Tumor-infiltrating lymphocytes (TILs) are lymphocytes that have moved from the blood into the tumor. Typically, TILs consist primarily of CD8+ cytotoxic T cells and CD4+ helper T cells. TILs are able to recognize and attack tumor cells so one therapeutic strategy is to isolate TILs from a tumor, expand them by stimulating with IL-2 and reintroduce the expanded population to attack the tumor. Clinical trials using TIL expansion therapies are ongoing in multiple tumor types including head and neck squamous cell cancers, cervical cancer, breast cancer, colorectal cancer and metastatic melanoma[14]. However, this therapeutic approach is limited by the presence of TILs in the tumors and several tumor indications are not amenable to this approach.

Engineered T cell therapy is an approach that is used when TILs are not present in the tumor tissue or microenvironment. In this approach, T cells are isolated from tumors and then engineered via retroviral or lentiviral transduction to express T cell receptors that recognize tumor specific antigens[15]. The T cell receptors are heterodimers consisting of alpha and beta subunits. The T cell receptor expressing cells bind to the major histocompatibility complex (MHC) on antigen presenting cells.

CAR-T cells are engineered T cells that do not require binding to MHCs expanding the range of target recognition[16]. Chimeric antigen receptors have an extracellular single chain Fv (variable fragment) domain that recognizes tumor associated antigens, and an intracellular domain derived from the TCR complex that triggers downstream signaling. Currently, there are two CAR-T cell therapies that have been approved by the FDA:

- Axicabtagene ciloleucel (Yescarta®): a CD19-targeting CAR T cell immuno therapy that is approved for subsets of patients with relapsed or refractory large B cell lymphoma
- Tisagenlecleucel (Kymriah®): a CD19-targeting CAR T cell immunotherapy; approved for subsets of patients with relapsed or refractory large B cell lymphoma and children and young adults with acute lymphoblastic leukemia (ALL).



One limitation of CAR-T cells is that they can only recognize cell surface antigens so if the tumor cells lose expression of the target antigen, the CAR-T cell therapy would not be effective[17]. Therefore, researchers are focusing on developing multiple generation of CAR-T cells expressing various target antigens. The next generation of CAR-T cells are being engineered to be safer and more efficacious.

CAR-T cell therapy has been associated with severe side effects that have been fatal in some cases, so there is active research to make CAR-T safer using a controlled activation of CAR-T or combining receptors from NK and T cells[18]. Another approach is to develop CAR-T therapies using gamma delta T cells that are rare T cells that selectively attack tumor cells.

Engineered NK cells are being investigated as an alternative to CAR-T therapies[19]. Autologous CAR-T therapies are cumbersome and limit large-scale clinical use but allogeneic T cells are not an option due to graft versus host disease. However, allogeneic NK cells do not cause graft vs host disease so they are a more attractive option and also have a good safety profile. However, the efficacy of CAR-NK cells is modest especially in the immunosuppressive tumor microenvironment so there is active research ongoing to improve engineered NK mediated tumor killing[19]. Given the rapid advances in the NK cell biology space, it is likely that safe and efficacious engineered NK cells will be added to the adoptive cell therapies toolbox.

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